Supplemental Figures

Figure S1

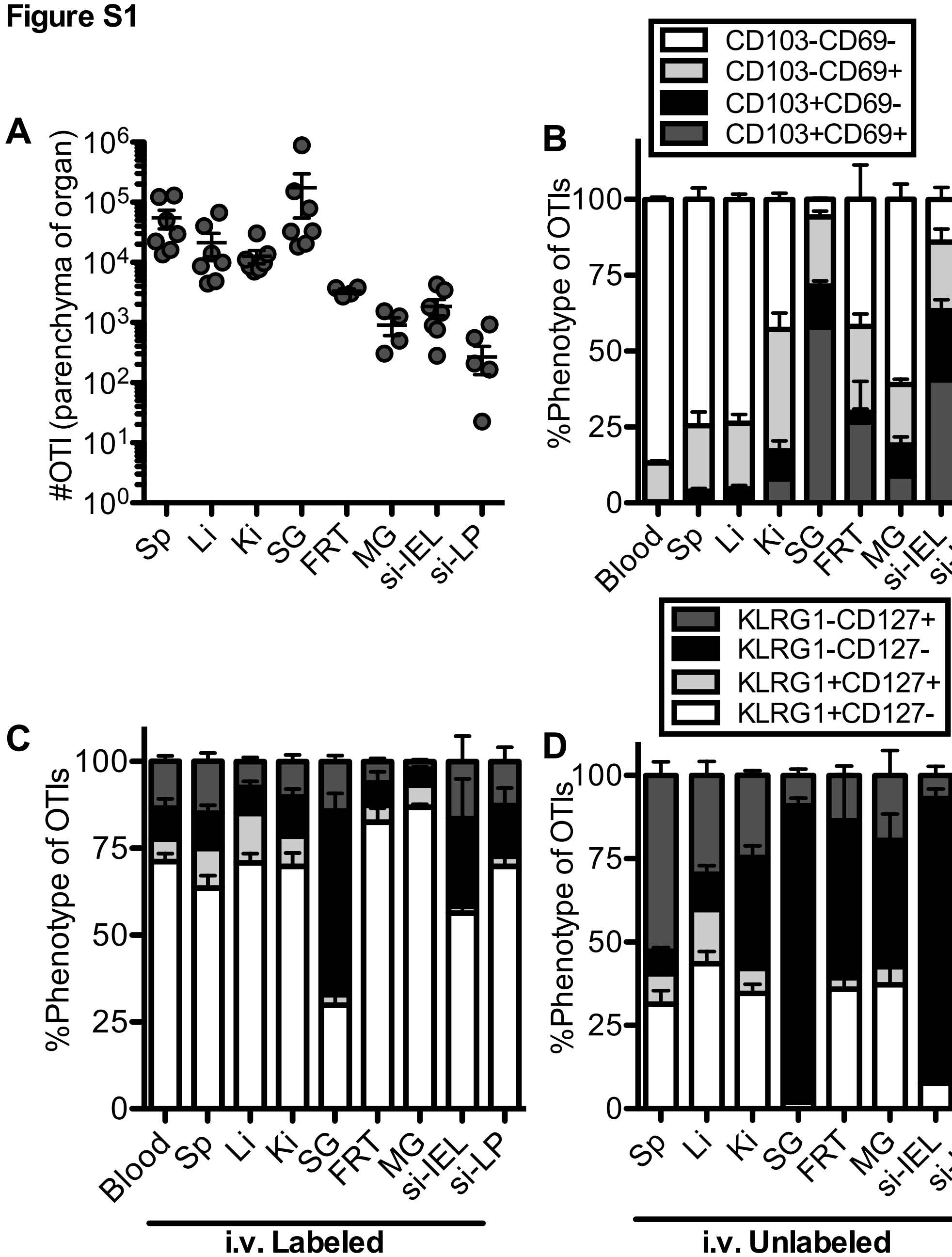

Figure S1 (Related to Figure 1): OT-Is in the parenchyma of organs display T_{RM} phenotype rather than an effector phenotype. (A) Absolute numbers of OT-Is from Figure 1 protected from i.v. staining within each organ. (B) OTIs in the unlabeled fraction of each organ were analyzed for expression of CD103 and CD69. Shown is the frequency of the indicated phenotype among OT-Is. (C, D) OT-Is that were either labeled or unlabeled with i.v. injected anti-CD8α antibody were analyzed for expression of KLRG-1 and IL-7Rα (CD127). Frequency of the indicated phenotype among OT-Is within the i.v. labeled (C) or unlabeled (D) fraction of each organ. Results are a combination of two independent experiments (n=4-7). Error bars represent the standard error of the mean.

Figure S2

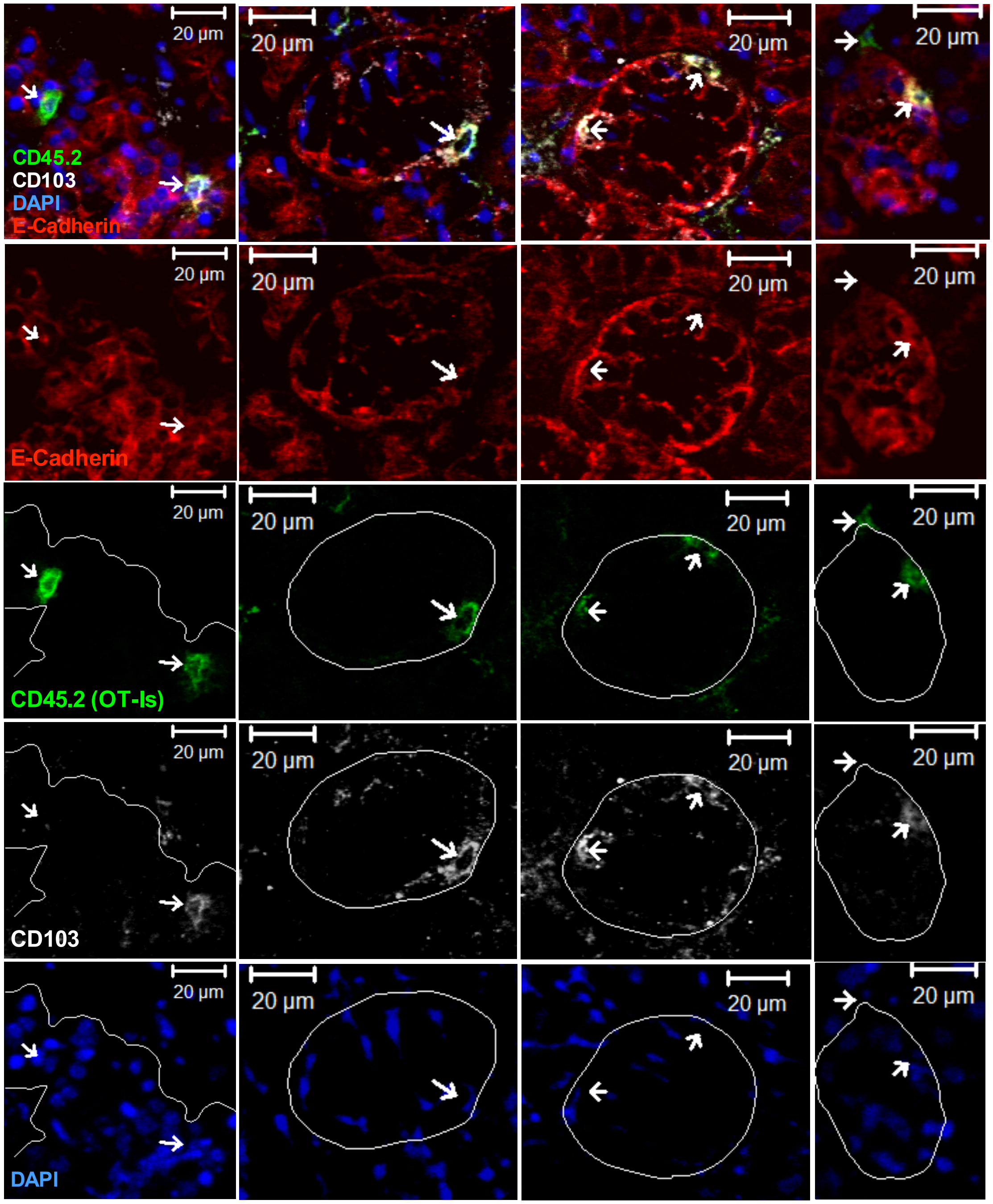

Figure S2 (Related to Figure 1): MCMV specific cells adopt an intraepithelial localization salivary gland. Shown are OT-Is (identified by CD45.2 expression, shown in green and marked by arrows) with and without CD103 expression (shown in white) as determined by immunofluorescent staining of the salivary gland >12 weeks after adoptive transfer and infection. OT-Is were frequently embedded in the epithelium (identified by E-cadherin, shown in red) in structures that are consistent with acinii and ducts. OT-Is that were not intraepithelial were frequently in close contact with epithelial structures. Overlays in bottom three rows mark the boundaries of epithelial structures. DAPI staining of the nucleus is shown in blue. Data is representative of four mice.

Figure S3

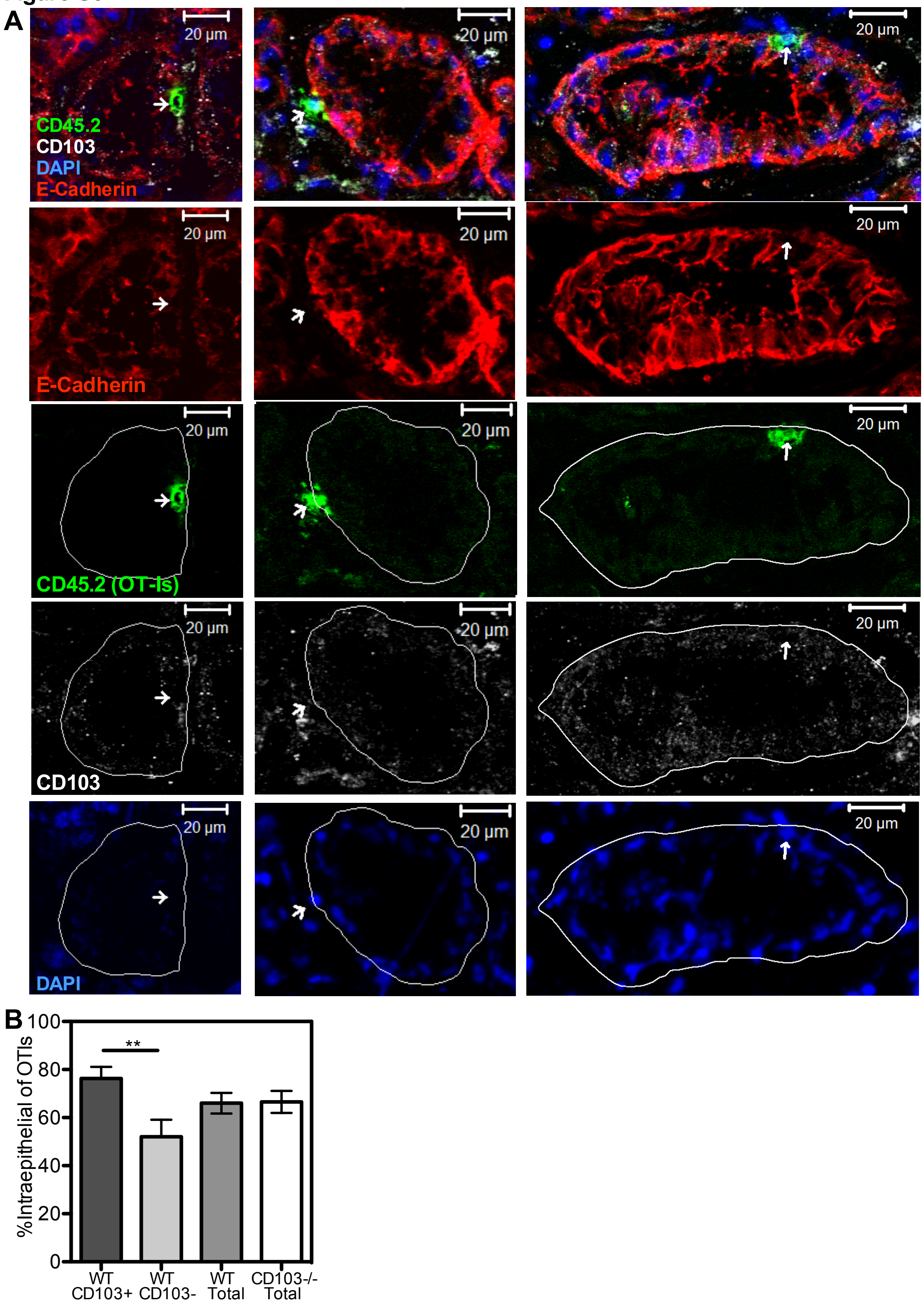
A
20 µm
CD45.2
CD103
DAPI
E-Cadherin
E-Cadherin
CD45.2 (OT-Is)
CD103
DAPI
B
%Intraepithelial of OTIs
100
80
60
40
20
0
**
WT CD103+
WT CD103-
WT Total
CD103-/- Total

Figure S3 (Related to Figure 2): CD103 expression is not required for intraepithelial localization of MCMV-specific CD8s in the salivary gland. As described in Figure 2, wild type or CD103-/- OT-Is were adoptively transferred and challenged by MCMV-OVA. (A) The localization of OT-Is in the salivary gland was examined by immunofluorescent staining >12 weeks post infection. CD103-/- OT-Is (identified by CD45.2 expression, shown in green and marked with arrows) have a distribution similar to wild type OT-Is and are readily found embedded in the epithelium (identified by E-cadherin, shown in red). CD103 staining is shown in white and DAPI staining of the nucleus is shown in blue. Overlays in bottom three rows mark the boundaries of epithelial structures. Data are representative of three mice. (B) Shown is the percent of OT-Is that were in embedded within the epithelium for wild type OT-Is with or without expression of CD103 and total wild type OT-Is compared to total CD103-/- OT-Is. Intraepithelial cells were defined as cells that were fully surrounded by E-cadherin-expressing cells. Data are representative of 3 mice with CD103-/- OT-Is and 4 mice with wild-type OT-Is. Statistical significance was measured by paired student’s t-tests (*p<.05, **p<.01, ***p<.001).

Figure S4

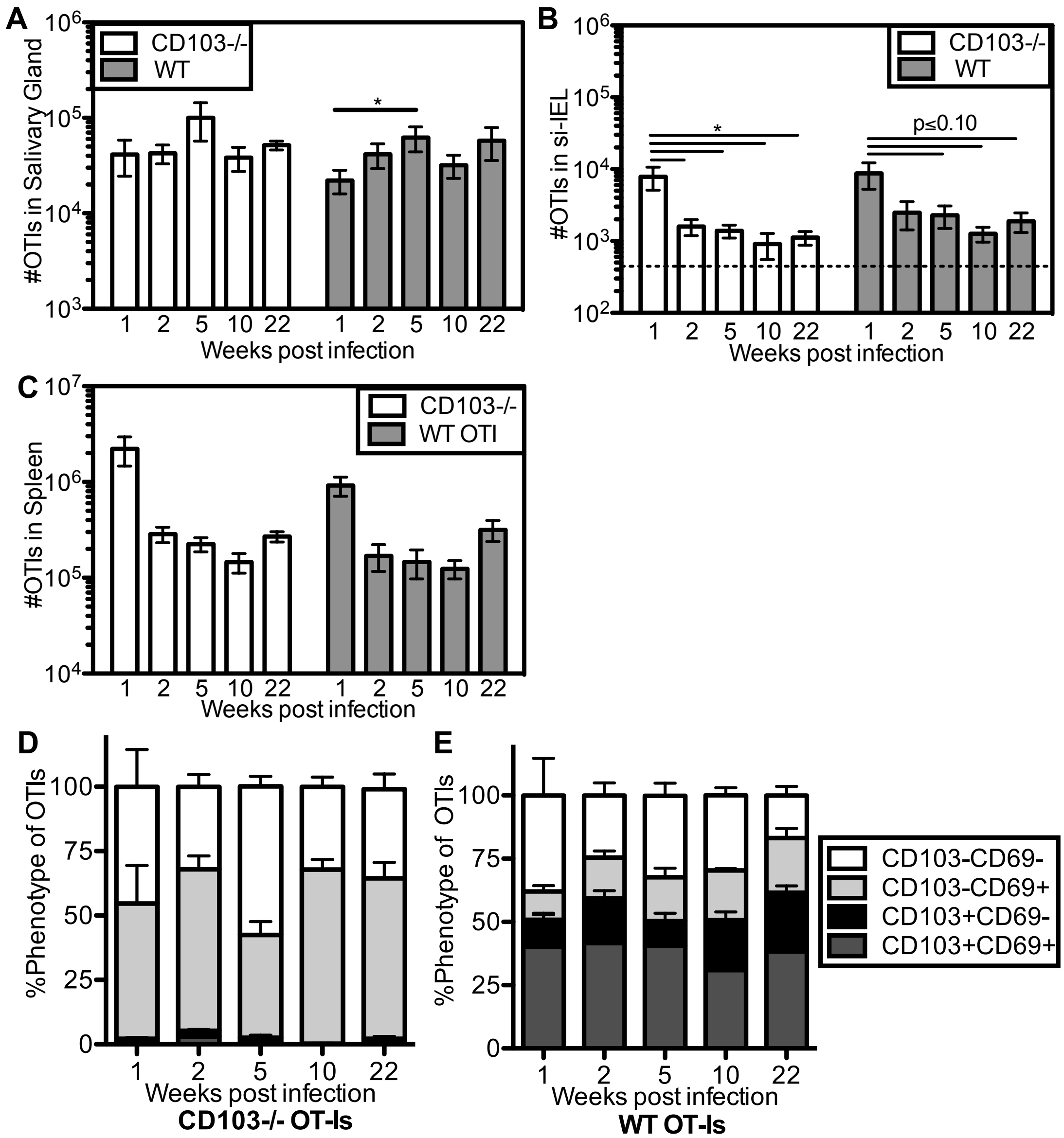
A
#OTIs in Salivary Gland
CD103-/-
WT
Weeks post infection
B
#OTIs in si-IEL
p≤0.10
C
#OTIs in Spleen
WT OTI
D
%Phenotype of OTIs
CD103-/- OT-Is
E
WT OT-Is
CD103-CD69-
CD103-CD69+
CD103+CD69-
CD103+CD69+

Figure S4 (Related to Figure 2): Maintenance and phenotype of wild-type and CD103-/- OT-Is in the spleen and small intestine IEL fraction. As in Figure 2, wild type and CD103-/- OT-Is were mixed together in the same mice, challenged with MCMV-OVA and cohorts were sacrificed at different times post infection. Absolute numbers of WT and CD103-/- OT-Is in the salivary gland (A) small intestine IEL (B) and spleen (C) over time. Dotted line in B indicates the limit of detection. (D, E) Frequency of the indicated phenotype within CD103-/- OT-Is (D) and WT OT-Is (E) in the small intestine IEL over time. Results are combined from two independent experiments (n=7). Error bars represent the standard error of the mean. Statistical significance was measured by paired student's t-tests (*p<.05, **p<.01, ***p<.001).

Figure S5

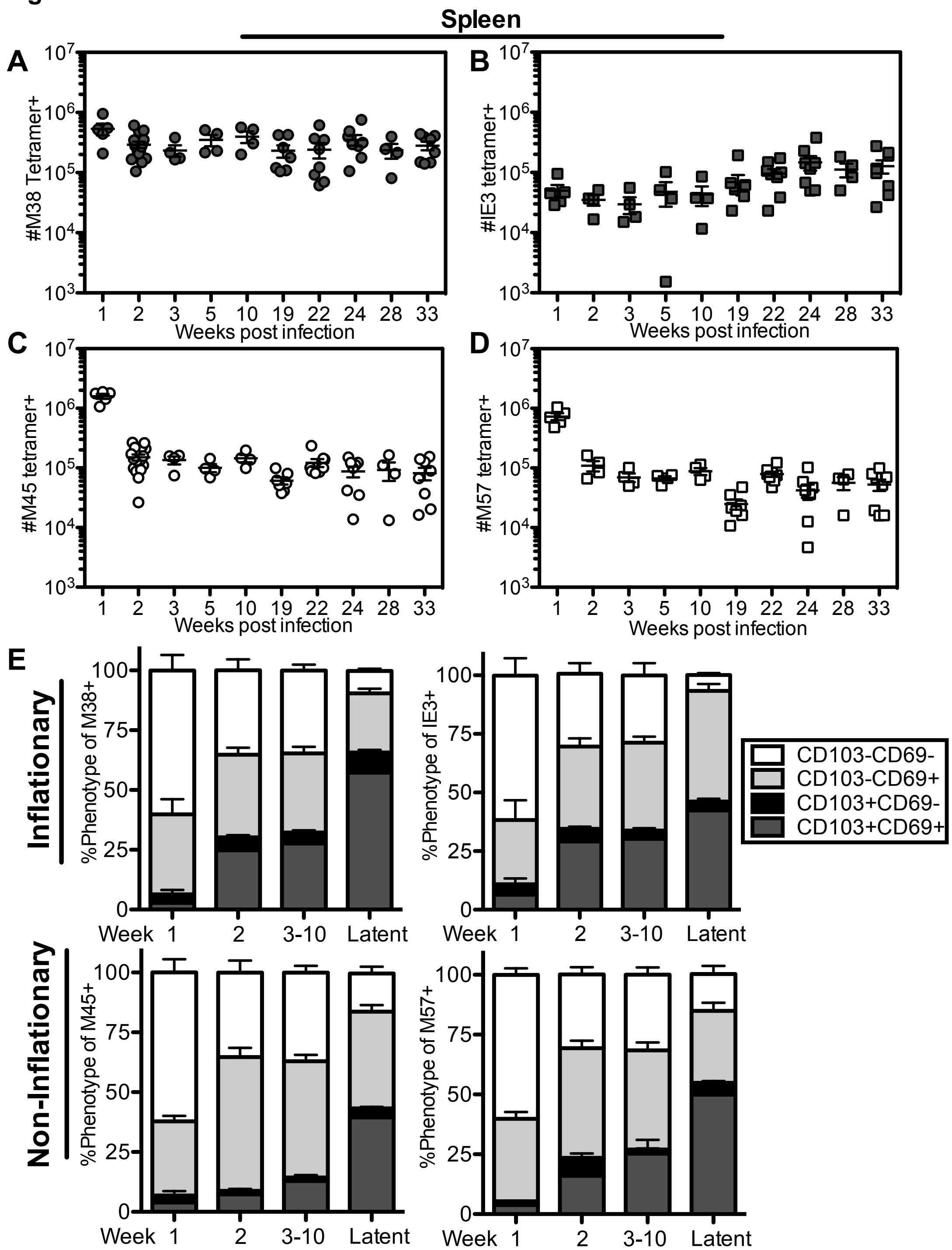
Spleen
A
#M38 Tetramer+
Weeks post infection
1 2 3 5 10 19 22 24 28 33
B
#IE3 tetramer+
Weeks post infection
C
#M45 tetramer+
Weeks post infection
D
#M57 tetramer+
Weeks post infection
E
Inflationary
Non-Inflationary
%Phenotype of M38+
%Phenotype of IE3+
%Phenotype of M45+
%Phenotype of M57+
Week 1 2 3-10 Latent
CD103-CD69-
CD103-CD69+
CD103+CD69-
CD103+CD69+

Figure S5 (Related to Figure 3): Inflationary CD8s are preferentially maintained in the salivary gland during the latent stages of infection. Absolute numbers of CD8s in the spleen specific for inflationary epitopes M38(A) and IE3 (B) and non-inflationary epitopes M45 (C) and M57 (D). Data are from the same animals described in Figure 3. (E) Frequency of the indicated phenotype among antigen specific CD8s at different times post infection. Data is combined from 7 independent experiments (n=4-16 per time point).

Figure S6

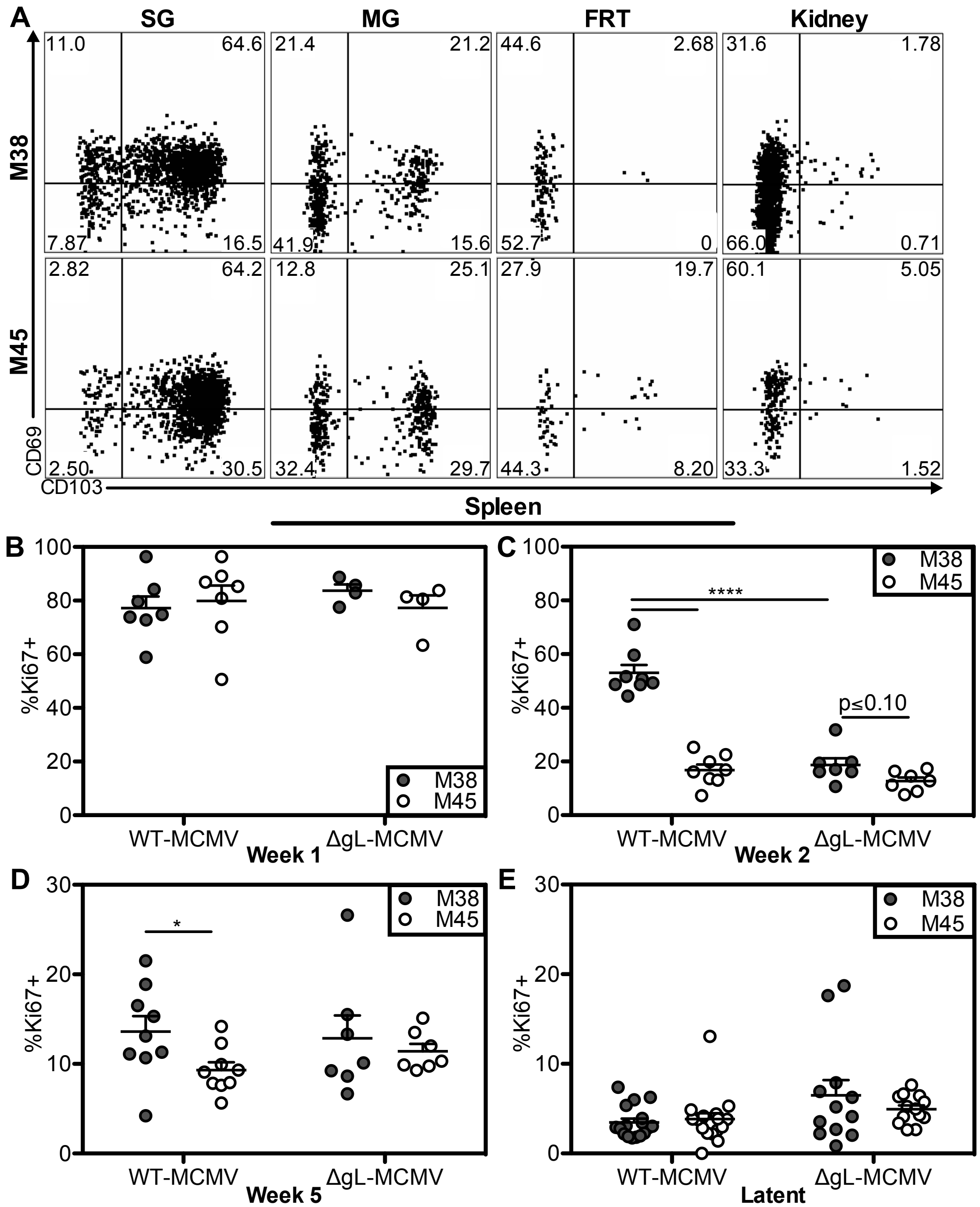
A
SG
MG
FRT
Kidney
M38
M45
CD69
CD103
11.0
64.6
7.87
16.5
21.4
21.2
41.9
15.6
44.6
2.68
52.7
0
31.6
1.78
66.0
0.71
2.82
64.2
2.50
30.5
12.8
25.1
32.4
29.7
27.9
19.7
44.3
8.20
60.1
5.05
33.3
1.52
Spleen
B
C
D
E
%Ki67+
M38
M45
WT-MCMV
ΔgL-MCMV
Week 1
Week 2
Week 5
Latent
****
p≤0.10
*

Figure S6 (Related to Figure 4): Viral replication in the salivary gland does not drive formation or replication of MCMV specific T_{RM} in the salivary gland. Mice were infected with spread defective ΔgL-MCMV and sacrificed >12 weeks post infection. Shown are representative FACS plots of CD103 and CD69 expression on M38-specific (top) and M45-specific (bottom) CD8s in the salivary gland, mammary gland, female reproductive tract and kidney. Frequency of Ki67 expression among antigen specific CD8s in the spleen at day 7 (B) day 14 (C) day 35 (D) or at latent times (E) post infection with wild type MCMV or ΔgL-MCMV. Error bars represent the standard error of the mean.

Figure S7

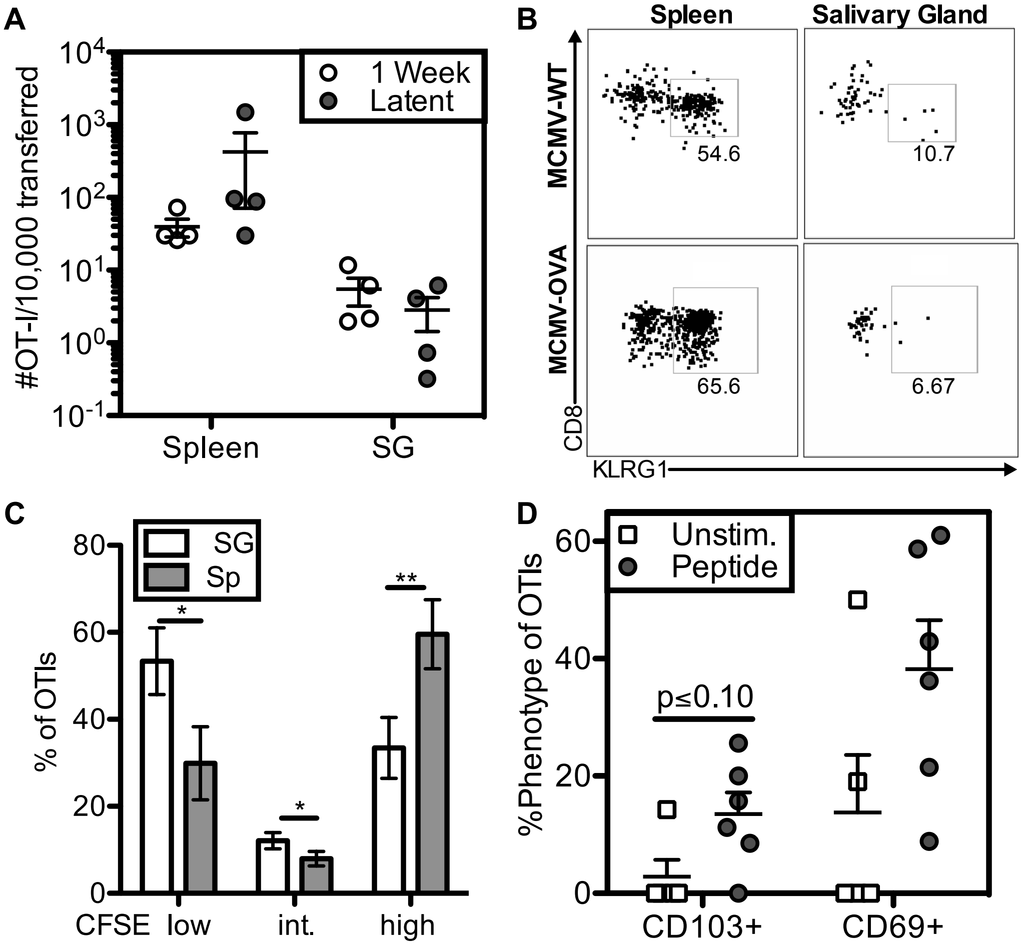
A
1 Week
Latent
#OT-I/10,000 transferred
Spleen
SG
B
Spleen
Salivary Gland
MCMV-WT
MCMV-OVA
54.6
10.7
65.6
6.67
CD8
KLRG1
C
SG
Sp
% of OTIs
CFSE
low
int.
high
D
Unstim.
Peptide
%Phenotype of OTIs
p≤0.10
CD103+
CD69+

Figure S7 (Related to Figure 7): Inflationary CD8s can become T_{RM} in the salivary gland during latent infection. OT-Is were isolated from the spleens of mice at either 1 week or >12 weeks after MCMV-OVA infection and transferred into congenic naive mice. (A) Number of OT-Is in the indicated organ of naive recipients per 10,000 transferred OT-Is. (B) Representative FACS plots of KLRG1 expression on transferred OT-Is in the spleen and salivary gland 2-4 weeks after transfer into MCMV-WT or MCMV-OVA latently infected mice. (C) Frequency of donor OT-Is expressing high (undiluted CFSE intensity), low (fully diluted CFSE) and intermediate levels of CFSE in the spleen and salivary gland of MCMV-OVA latently infected recipients. (D) Frequency of CD103 and CD69 expression on OT-Is after in vitro stimulation and transfer into naive recipients. Error bars represent the standard error of the mean. Statistical significance was measured by unpaired student's t-tests (*p<.05, **p<.01, ***p<.001).

Supplemental Materials

Primers for QPCR were taken from the following: Gapdh and TGFβ1(Stock et al., 2014), TNFα (Fontenot et al., 2003), and IL-33 (Li et al., 2014).

Primer	Sequence
Gapdh Forward	CCA GGT TGT CTC CTG CGA CTT
Gapdh Reverse	CCT GTT GCT GTA GCC GTA TTC A
TGFβ1 forward	ACC ATG CCA ACT TCT GTC TG
TGFβ1 reverse	CGG GTT GTG TTG GTT GTA GA
TNFα forward	ACT GAA CTT CGG GGT GAT CG
TNFα reverse	TGA TCT GAG TGT GAG GGT CTG G
IL-33 forward	ACT ATG AGT CTC CCT GTC CTG
IL-33 reverse	ACG TCA CCC CTT TGA AGC

Supplemental References

Fontenot, J.D., Gavin, M.A., and Rudensky, A.Y. (2003). Foxp3 programs the development and function of CD4+CD25+regulatory T cells. Nature immunology *4*, 330-336.

Li, D., Guabiraba, R., Besnard, A.-G.e., Komai-Koma, M., Jabir, M.S., Zhang, L., Graham, G.J., Kurowska-Stolarska, M., Liew, F.Y., McSharry, C., and Xu, D. (2014). IL-33 promotes ST2-dependent lung fibrosis by the induction of alternatively activated macrophages and innate lymphoid cells in mice. J Allergy Clin Immunol *134*, 1422-1432.

Stock, A.T., Smith, J.M., and Carbone, F.R. (2014). Type I IFN suppresses Cxcr2 driven neutrophil recruitment into the sensory ganglia during viral infection. The Journal of experimental medicine *211*, 751-759.